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Mini-round box as standardized sampling method for orthopterans in alpine and subalpine grasslands: a field study to highlight strengths and weaknesses

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Abstract

Orthopterans are known as suitable ecological indicators in grassland habitats, with their community composition providing useful information about the environmental consequences of management actions, ecological processes, or climate change. However, community studies often require the collection of both species richness and abundance data, which are difficult to obtain for these insects without a proper sampling strategy in certain environmental and population density conditions. In general, box quadrats with high sides (≥ 1 m^2) represent a valuable method to assess orthopteran assemblages in open habitats, although their big size might be inappropriate for challenging environments, such as high-elevation alpine grasslands. For this reason, in this paper the effectiveness of a smaller (0.16 m^2) and handy (circular-shaped) version of the box quadrat sampling device (hereafter called "mini-round box") is tested in the field. Then, through a Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis, the positive and negative features of this sampling method are highlighted, focusing in particular on the alpine and subalpine grassland context. Overall, the mini-round box strategy showed a good potential as a handy, easy, cheap, and standardized sampling method, but serious shortcomings in species detection have been observed (i.e. 47% of species undetected in average). A number of valuable strengths and interesting opportunities are counteracted by serious weaknesses and significant threats, which need to be carefully evaluated when planning a sampling design involving orthopterans as indicators. Some solutions to improve the mini-round box accuracy are suggested, perhaps encouraging the performance of biodiversity monitoring and ecological studies on orthopterans in even challenging grassland ecosystems.

Key Words

Alpine habitats, community composition, orthoptera, qualitative survey, sampling accuracy, SWOT analysis

Introduction

Biodiversity monitoring is an effective tool to assess ecosystem health, to address conservation priorities, and to evaluate the success/failure of environmental policies and management (Schmeller et al. 2017). However, its effectiveness strictly depends on the implementation of accepted, rigorous, repeatable, and standardized measurements, essential to collect high-quality data comparable in space and time (Lovett et al. 2007). In this context, when monitoring biodiversity at ecosystem level, the number and relative abundance of species are commonly used metrics to assess biotic assemblages (Noss 1990). Indeed, these parameters provide compositional data which often reflect the structure (e.g. habitat, land use, etc.) and function (e.g. biogeochemical cycles, trophic interactions, etc.) of the investigated natural systems. This is particularly true when indicator taxa are used as monitoring targets, due to their sensitivity to environmental changes and ecological disturbances (Hilty and Merenlender 2000), especially if they allow for cost-effective measurements (Carignan and Villard 2002).

Orthopterans are known as suitable ecological indicators in grassland habitats (e.g. Báldi and Kisbenedek 1997; Bazelet and Samways 2011; Fartmann et al. 2012). In particular, their indicator value is due to their relative ease in species detection and identification in the field (even by listening their species-specific stridulation sounds), their specific habitat requirements, and their inter-specific variability in functional traits, including for instance size, reproductive strategy, fertility, dispersal capacity, diet, and climatic niche (Moretti et al. 2013; Ancillotto and Labadessa 2024). More in detail, the sensitivity of these insects to local vegetation structure and microclimate affects their community composition (e.g. Guido and Chemini 2000; Gardiner et al. 2002; Gardiner and Hassall 2009; Kenyeres et al. 2019), which in turn can provide useful information about the environmental consequences of grassland management actions, ecological processes, or climate change. Notwithstanding, the collection of quantitative orthopteran data is not an easy task for grassland ecologists, since a unique, exhaustive, standardized, and comparable sampling technique is still lacking (Gardiner et al. 2005).

In their review, Gardiner et al. (2005) identified sward height and population density as the main constraints for quantitative orthopteran sampling in grassland habitats. These authors suggest the implementation of "flushing" techniques (e.g. open quadrats, transect counts, etc.) in case of short vegetation (< 50 cm) and low population densities (< 2 ind./m^2). On the other hand, "capture" strategies (e.g. box quadrats, sweep netting, etc.) are proposed when a high number of individuals occur in the area to be surveyed (> 2 ind./m^2). Sampling orthopterans in tall vegetation (> 50 cm) to obtain quantitative data is indicated as the most challenging situation, especially in cases of high population densities.

Since in alpine and subalpine grasslands the height of herbaceous plants rarely exceeds 50 cm, these habitats provide favourable conditions for quantitative studies on orthopterans. However, significant changes in population density may occur even within a single study area, depending on habitat, elevation, aspect, microclimatic conditions or other local factors. For instance, abundant orthopteran populations may occur in most South-exposed grasslands at low elevation, while a limited number of individuals is often found at high elevations (especially on North-facing slopes). For this reason, the "capture" sampling methods have been largely preferred in previous research in the alpine context, in order to successfully manage density constraints. In particular, sweep netting (Guido and Chemini 2000; Fabriciusová et al. 2011; Illich and Zuna-Kratky 2022), biocenometers (Klein et al. 2020; Kurtogullari et al. 2020), and box quadrats with high sides (Marini et al. 2009a, 2009b, 2012; Löffler and Fartmann 2017) are the most commonly used sampling strategies to quantify orthopteran species richness and abundance in alpine and subalpine grasslands, at least in recent years.

While sweep netting may be affected by some shortcomings in terms of standardization (O'Neill et al. 2002; Gardiner et al. 2005), the suitability of box quadrats for orthopteran community assessments was recently confirmed, even proposing this sampling strategy as standard method for systematic long-term orthopteran monitoring in European grasslands (Fartmann et al. 2024). Box size is reported as a key issue to achieve accuracy and exhaustivity standards, highlighting the high risk of density underestimates when a sampling unit smaller than 1 m^2 is used (Badenhausser et al. 2009). Conservatively, Fartmann et al. (2024) even recommend the use of a 2 m^2-box quadrat in standardized orthopteran samplings in open habitats.

Nevertheless, several logistic and environmental constraints have to be taken into account when considering orthopteran research in challenging environments, such as high-elevation alpine grasslands. For instance, sampling points may require a long approach by foot (i.e. involving equipment transport issues); while operator's skill and mobility may be limited on steep slopes, especially if a big and heavy device has to be handled for samplings. Therefore, the use of an unhandy 1 m^2- (or even bigger) sampling box might be inappropriate to study orthopteran species richness and relative abundance in such particular circumstances. The use of a small (i.e. < 1 m^2) and more manageable sampling box has been already experimented by some authors investigating orthopteran communities in alpine and subalpine grasslands (0.33 m^2, Marini et al. 2008; 0.18 m^2, Battisti et al. 2016 and Giuliano et al. 2017; 0.50 m^2, Löffler and Fartmann 2017), also introducing a modified version of the box quadrat with high sides, based on a circular-shaped box (hereafter called "mini-round box"; Marini et al. 2008; Battisti et al. 2016; Giuliano et al. 2017).

Focusing on this latter version, mini-round boxes appear as a potential trade-off between the application of a valuable survey method (i.e. box-based samplings) and the convenient use of a handy sampling device. However, a specific pros and cons analysis concerning the implementation of this method in the alpine context is still lacking, preventing ecologists to make an informed choice when planning orthopteran samplings in alpine and subalpine grasslands.

For this reason, in this paper a Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis on the mini-round box method is compiled, benefiting from field data collected in alpine and subalpine grasslands and considering the available literature. The general purpose of this research is to provide grassland ecologists with the necessary information to possibly answer this question: is the mini-round box sampling strategy suitable for my research purposes?

In order to gather information for the SWOT analysis, two secondary aims were pursued in this study. First, the mini-round box accuracy was assessed in the field from a qualitative point of view, comparing the orthopteran species list resulting from mini-round box surveys in each site with a reference checklist obtained in simultaneous visual and acoustic transects. In this case, a species richness underestimation in mini-round box samples was highly expected, since the sampling unit tested here (0.16 m^2) was significantly smaller than those recommended in literature for box-based samplings (i.e. ≥ 1 m^2; Badenhausser et al. 2009; Fartmann et al. 2024). Therefore, as a further specific objective, the effects of grass height, study site, and

species' mobility on sampling accuracy were explored, in order to identify possible factors (other than box size) limiting the mini-round boxes performances.

Methods

In this paper, the mini-round box sampling strategy is evaluated using a Strengths-Weaknesses-Opportunities-Threats (SWOT) approach. SWOT is a tool deriving from business literature, usually applied by organizations and companies for strategic planning and management (Gürel and Tat 2017), but extendable to a wide array of decision-making processes, including environmental management and assessment (e.g. Scolozzi et al. 2014; Bull et al. 2016; Jetoo and Lahtinen 2021). Basically, SWOT is used to facilitate a realistic, fact-based, and data-driven look towards the achievement of a specific goal. In this case, the "goal" is the evaluation of mini-round boxes as an effective sampling method to investigate orthopteran communities in alpine and subalpine grassland habitats.

The SWOT analysis is typically performed considering two dimensions: internal and external. The former includes organizational factors, usually controlled by the company/operator (i.e. strengths and weaknesses), while the latter encompasses often out-of-control environmental components (i.e. opportunities and threats) (Sarsby 2016). In addition, these factors can be further distinguished in helpful (i.e. contributing to the goal achievement: strengths and opportunities) and harmful (i.e. hindering the goal achievement: weaknesses and threats) (Sarsby 2016). Accordingly, results are usually summarized in a 2×2 matrix.

Although more analytical versions exist (Chang and Huang 2006), in this study the SWOT framework is applied to simply provide a clear list and categorization of the positive and negative factors involved in the performance of a mini-round box sampling strategy to survey orthopteran communities in alpine and subalpine grasslands. In particular, the intrinsic technical features of the sampling method were considered either as strengths or weaknesses (i.e. internal factors), while environmental, strategic, and ecological issues were evaluated as opportunities or threats (i.e. external factors).

In order to inform the SWOT evaluation process, a field study based on mini-round box orthopteran samplings has been carried out in two sites of the Western Italian Alps (Cottian Alps, Piedmont): the Troncea Valley (Pragelato, TO; 44.9578°N, 6.9540°E) and Rocca Bianca (Oncino, CN; 44.6649°N, 7.1610°E). Both sites are included within a protected area (the Val Troncea Natural Park and the Monviso Natural Park respectively). Surveys were performed along 12 transects (200 m in length), eight in the Troncea Valley and four at Rocca Bianca, placed in subalpine and alpine grasslands between 1590 and 2590 m a.s.l. Except for 4 ungrazed transects in the Troncea Valley, all sampling stations were managed by cattle grazing during the study period, producing changes in grass height throughout the summer. Data were collected fortnightly in each transect (in order to allow an adequate number of repetitions in the sampling season), between mid-July and the end of September 2021 (5 sessions×12 transects), in sunny and calm-wind days between 9:00 A.M. and 6:00 P.M. At Rocca Bianca, each sampling session was usually completed in a single day, while in the Troncea Valley two subsequent days were required to investigate all transects.

In each transect, the orthopteran community has been investigated by applying simultaneously two sampling methods: the mini-round box strategy and a qualitative survey, combining visual and acoustic census (Mourguiart et al. 2020). In particular, mini-round box samplings were performed using a standard sampling unit of 0.16 m^2, identified in the field by means of a cylinder of 45 cm in diameter and 50 cm in height (Fig. 1). This folding sampling device was also provided with a lid to prevent the escape of the sampled individuals, while a graduated ruler was included inside to allow grass height measurements. The cylinder has been randomly thrown into the grass 60 times along each 200 m-transect (30 on the way there, 30 on the way back; overall corresponding to a sampling area of 9.6 m^2 per transect), paying attention to ensure independence among sample units (i.e. in terms of distance: at least 10 steps) while distributing them as homogeneously as possible on the whole transect length. This number of repetitions per transect was selected to correspond to the minimum sample size area suggested by Ingrisch and Köhler (1998) to ensure orthopteran species richness saturation with box quadrat surveys (i.e. 9 m^2).

In each sampling unit, all the orthopterans were searched, identified following Sardet et al. (2015) and Iorio et al. (2019), and after released (i.e. qualitative data only). In few cases (e.g. genus *Anonconotus*), species identification was achieved by examining titillators' morphology, thus requiring the suppression of the sampled individuals. In addition, grass height was measured within the cylinder (i.e. averaged on 0.16 m^2) each time the sampling unit was inspected, thus allowing for the calculation of an estimated mean vegetation height along transects.

The box size used in this research (0.16 m^2) is similar to the smallest one previously used for orthopteran studies in the alpine context (0.18 m^2; Battisti et al. 2016; Giuliano et al. 2017), following an opposite strategy as compared to the recommendations of Badenhausser et al. (2009) and Fartmann et al. (2024). Bigger box sizes (i.e. between 0.16 and 1 m^2) were not included in the sampling design, assuming a progressive decline in sampling accuracy with a decreasing sampling unit area (Badenhausser et al. 2009).

While performing mini-round box surveys, all the orthopteran species seen and heard in a 10 m-buffer from the operator were recorded, using the recordings provided by Odé (2012) as reference for the identification of stridulations. Also in this case, no abundance data were collected, due to the complexity of estimating the number of individuals in the field basing on stridulations only, and on non-standardized observations.

This sampling design resulted in two presence/absence matrices: one containing the mini-round box data only, and another one merging mini-round box data with those obtained in the qualitative survey (hereafter "combined

survey"). From these matrices, species richness values were extracted for each monitoring method, in each data collection event for each sampling station. In this framework, the orthopteran community resulting from the combined survey was considered as a proxy of the whole assemblage occurring in each sampling station in each data collection event. Therefore, the combined survey results were used as reference to assess mini-round box sampling accuracy, assuming that the combination of different sampling methods would increase the probability of detecting all the species occurring in a given area, following the results obtained by Mourguiart et al. (2020).

The mini-round box accuracy was assessed at two different levels: species richness and community composition. At first, possible differences in term of species richness between mini-round box and combined surveys were explored by means of the Wilcoxon Signed-Rank Test (Wilcoxon 1945), considering the values collected in each transect per sampling session as paired samples (i.e. mini-round box vs combined survey). Then, the composition of the two orthopteran assemblages resulting from the two survey methods was compared, both graphically (PCoA, Principal Coordinate Analysis; using the Jaccard dissimilarity index, as recommended for presence/absence data) and statistically (PERMANOVA; Anderson 2001). This latter method evaluates whether the observed differences between two communities deviate or not from a random distribution, examining the possible occurrence of a statistical significance through 9999 permutations. Transect and sampling session were specified as strata, in order to correctly deal with the spatial and temporal autocorrelation of the data.

In order to evaluate the effect of species' mobility on mini-round box accuracy, each species was classified into one of three broad mobility classes, following Reinhardt et al. (2005) and Marini et al. (2010): low, moderate and highly mobile species (Table 1). In the few cases of species not reported by these authors, a mobility class was assigned empirically by the authors, mainly basing on wing development (i.e. apterous species as sedentary). A mean community mobility index was calculated for each data collection event in each sampling station, considering mini-round box and combined survey separately. As for species richness, possible differences in term of species mobility between the two survey methods were investigated by means of the Wilcoxon Signed-Rank Test (Wilcoxon 1945) for paired samples.

The mini-round box representativeness of the orthopteran community was evaluated from a qualitative point of view, calculating in each transect per sampling session the proportion (%) of species detected, using the combined surveys data as reference (i.e. 100%). Then, the effect of the mean grass height along transects on mini-round box accuracy was tested with a Generalized Linear Mixed-Effects Model (GLMM). The model was run accounting for a Beta distribution, typically used when dealing with percentage data as dependent variable (Johnson et al. 1995; Salinas Ruíz et al. 2023), including the sampling session as random factor, in order to incorporate the temporal dependency among observations (Zuur and Ieno 2016). Conversely, the study site was included in the model as a fixed effect, due to its limited number of levels (n = 2), which may not provide accurate estimates of group-level variation (Gelman and Hill 2006; Harrison et al. 2018). Model assumptions were verified by plotting residuals versus fitted values and covariates, following the recommendations provided by Zuur et al. (2009).

Figure 1. Mini-round box. Mini-round box placed in a subalpine grassland at Rocca Bianca (Oncino, CN; Monviso Natural Park). The cylinder has a diameter of 45 cm, corresponding to a circular sampling unit of 0.16 m^2. Box sides are 50 cm in height.

All statistical analyses were performed with the software *R* (version 4.3.2; R Core Team 2023), using in particular the packages vegan (PCoA and PERMANOVA; version 2.6-4; Oksanen et al. 2022) and glmmTMB (Beta-GLMM; version 1.1.8; Brooks et al. 2017).

Results

Overall, the data collection performed to inform the SWOT evaluation process allowed the detection of 29 orthopteran species (20 in the Troncea Valley, 16 at Rocca Bianca). All of them were recorded in the visual and acoustic surveys, while a subset of 24 taxa was successfully sampled by means of the mini-round box method (Table 1). Accordingly, no species were detected exclusively with the mini-round box survey strategy.

The observed differences between mini-round boxes and combined survey in terms of number of species recorded proved to be statistically significant (Wilcoxon Signed-Rank Test: $V = 1711$; $p < 0.001$; Fig. 2A). In particular, the mean representativeness of mini-round boxes proved to be limited to the 53.0±21.4% of the species richness detected with the combined survey. Furthermore, the sampling method proved to condition the results also in terms of community composition (PERMANOVA: $F_{1,117} = 2.844$; $p < 0.001$; PCoA: Fig. 2B), especially because some species (*Oedipoda caerulescens*, *Omocestus viridulus* and *Myrmeleotettix maculatus* in the Troncea Valley; *Polysarcus denticauda*, *Tettigonia cantans*, *Nemobius sylvestris*, *Euthystira brachyptera*, *Omocestus haemorrhoidalis* and *Gomphocerus sibiricus* at Rocca Bian-

Table 1. Species list. List of the orthopteran species observed in the study area, indicating their presence/absence in each transect (N = 12) and sampling site (i.e. Troncea Valley and Rocca Bianca), merging the results of 5 sampling sessions. (**X**) indicates the taxa successfully observed with the mini-round box method, while (**O**) highlights the species detected only considering the visual and acoustic survey. In the column "M" the mobility index values for each species are reported (1 = low, 2 = moderate, 3 = high), in accordance with Reinhardt et al. (2005) and Marini et al. (2010). Nomenclature and taxonomic order follow Iorio et al. (2019).

Species	M	Troncea Valley								Rocca Bianca			
		1	2	3	4	5	6	7	8	9	10	11	12
Polysarcus denticauda (Charpentier 1825)	2									O			O
Tettigonia cantans (Fuessly 1775)	2									O			O
Decticus verrucivorus (Linnaeus 1758)	1			X	O	X	O						
Platycleis grisea (Fabricius 1781)	2					X							
Metrioptera saussuriana (Frey-Gessner 1872)	2									X	X	O	X
Bicolorana bicolor (Philippi 1830)	3		O	X	O		X						
Pholidoptera aptera (Fabricius 1793)	1											X	O
Anonconotus baracunensis Nadig 1987	1									X	X	O	X
Anonconotus occidentalis Carron and Wermeille 2002	1	X	X		X	X	X	X	X				
Nemobius sylvestris (Bosc 1792)	1									O		O	
Tetrix depressa Brisout de Barneville 1848	2			X		X							
Epipodisma pedemontana (Brunner von Wattenwyl 1882)	1	X	X		X	X	X	X	X				
Psophus stridulus (Linnaeus 1758)	1									O	X	O	O
Oedipoda caerulescens (Linnaeus 1758)	3			O									
Oedipoda germanica (Latreille 1804)	1			O	O	X							
Arcyptera (*Arcyptera*) *fusca* (Pallas 1773)	1		O	O	X	X	X	O	X				
Euthystira brachyptera (Ocskay 1826)	2		X	X	X		X			O			
Omocestus (*Omocestus*) *viridulus* (Linnaeus 1758)	2		O				O			X			X
Omocestus (*Omocestus*) *haemorrhoidalis* (Charpentier 1825)	1			X	O	X	X	X				O	
Stenobothrus cotticus Kruseman and Jeekel 1967	1								X				
Stenobothrus lineatus (Panzer 1796)	2									X	X	O	O
Stenobothrus nigromaculatus (Herrich-Schäffer 1840)	1	X	X	X	X	X	X	X	X				
Gomphocerus sibiricus (Linnaeus 1767)	2	X	X		X	X	O	X	X		O	O	
Myrmeleotettix maculatus (Thunberg 1815)	2			O	O	O		O	O				
Stauroderus scalaris (Fischer von Waldheim 1846)	3	X	X	X	X	X	X			O	X	O	
Pseudochorthippus parallelus (Zetterstedt 1821)	3									X	X	X	X
Chorthippus (*Chorthippus*) *dorsatus* (Zetterstedt 1821)	3			X						X	X	O	X
Chorthippus (*Glyptobothrus*) *apricarius* (Linnaeus 1758)	2	X	X	X	X	X	X						
Chorthippus (*Glyptobothrus*) *mollis* (Charpentier 1825)	3			X	O	X				O	X	X	

ca) were not detected with the mini-round box strategy throughout all the sampling season, in spite of their actual occurrence in the monitoring sites (Table 1).

Lastly, 12 (41.4%) of the orthopteran species detected in the study area are characterised by a low mobility, while 11 (37.9%) are moderate dispersers and 6 (20.7%) highly mobile species (Table 1). No differences between mini-round box and combined survey were observed in terms of mean mobility index at community level (Wilcoxon Signed-Rank Test: $V = 651.5$; $p = 0.234$; Fig. 2C). Similarly, the mean grass height along transects (ranging between 3.67 and 30.00 cm in the study area) proved to be statistically irrelevant for mini-round box accuracy (Beta-GLMM: Est. = 0.004; SE = 0.010; $z = 0.349$; $p = 0.727$; Fig. 2D). However, the model highlighted a significant effect of the study site on the mini-round box representativeness (Beta-GLMM: Est. = 0.818; SE = 0.164; $z = 4.972$; $p < 0.001$), with higher mean accuracy levels observed in the Troncea Valley (61.7±17.4% vs the 35.6±18.2% at Rocca Bianca; Fig. 2E).

Merging these outcomes with literature data and other practical issues, a SWOT matrix was compiled, including 9 factors as mini-round box strengths, 4 as weaknesses, 7 as opportunities, and 6 as threats (Table 2).

Discussion

The implementation of a mini-round box survey strategy in alpine and subalpine grasslands proved to involve a number of positive (i.e. strengths and opportunities) and negative (i.e. weaknesses and threats) factors, as summarized in Table 2.

Starting from strengths and opportunities, the mini-round box is a low-cost, handy and highly manageable device, ensuring a number of logistic advantages. First of all, the purchase or building of a mini-round box is quite inexpensive (< 50 € in this study). Then, its small dimensions (diameter 45 cm; 0.16 m^2) and light weight (≈ 1 Kg) facilitate the box use on steep and uneven slopes, also simplifying its transport towards less accessible sampling areas, as for instance the high-elevation alpine grasslands reachable only by foot. The use of a folding box (as performed in this study) would further increase its portability, even improving the sampling set up quickness (few seconds). All these advantages are clearer if the mini-round box features are compared with those of the bigger box quadrat proposed by Fartmann et al. (2024). Indeed, these authors suggest the use of a modular 1.41×1.41

m-device, weighting about 6 Kg, and requiring to be disassembled for transport, with an assembly and disassembly time of few minutes respectively. The total costs for the construction of this 2 m^2-box is about 300 €.

Another important positive feature of the mini-round box sampling strategy is its ease of use. To throw into the grass the sampling unit and the following search of orthopteran specimens in it are simple and quick procedures, easily performed by a single and even inexperienced operator. For instance, in this research a single trained worker was able to complete 60 mini-round box samples in about 30–45 minutes per transect, depending on orthopteran catch frequency. This is often important when a single operator has to visit several low accessible sampling sites within the same day in the alpine context. Unavoidably, the use of larger boxes would entail more setup (i.e. assembly/disassembly) and search time, the latter in order to ensure a complete survey of the whole sampling unit. In addition, more than one operator might be necessary, e.g. to check for escaped individuals as reported by Badenhausser et al. (2009).

As for other box-based sampling techniques, a major strength of mini-round boxes is their value as a standardized "capture" method (Gardiner et al. 2005). Indeed, this strategy allows for sampling in even dense orthopteran communities using a well-defined sample unit, with high box sides preventing the captured individuals to readily escape. This simplifies a lot the specimen search, identification, count, and collection, resulting in valuable species richness and abundance data. Moreover, the specimens' catch enables to identify both sex and life stage (i.e. adult or nymph) of the collected individuals, with multiple implications in ecology. The mini-round box technique is also suitable for random sampling (i.e. the box is randomly thrown into the grass), making this method (as other box-based surveys) particularly appropriate for ecological studies in grassland habitats.

A valuable feature of mini-round boxes is then their independence from grass height, as observed in this study. Indeed, the sampling accuracy with this method showed negligible changes in relation to the mean grass height along transects (at least in the 3.67–30.00 cm-range available in the study area), corroborating the overall suitability of this technique to investigate the orthopteran community across various alpine and subalpine grassland environments. For instance, a rather constant sampling accuracy can be assumed between grazed and non-grazed pastures, where grass height is one of the main ecological drivers

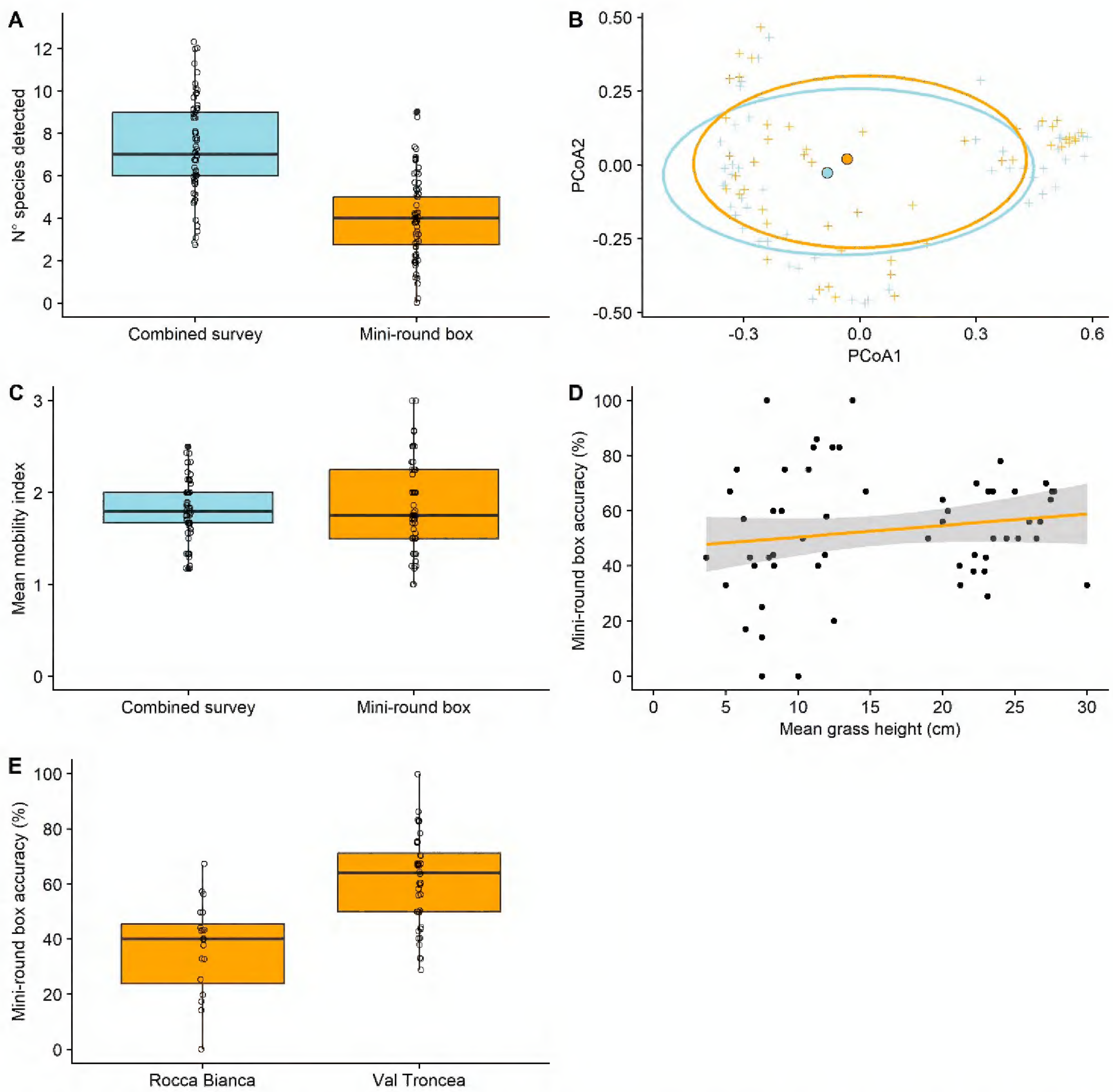

Figure 2. Graphic results. Plots representing: **A.** The different values of species richness observed with the mini-round box method and the combined survey (mini-round box + visual and acoustic survey); **B.** The differences in community composition resulting from the two sampling methods (PCoA; orange: mini-round box; light blue: combined survey); **C.** The mean mobility index of the orthopteran communities sampled with the two survey methods; **D.** The observed trend of mini-round box accuracy (%) in relation to the mean grass height along transects; **E.** The observed differences in terms of mini-round box accuracy (%) between the two study sites investigated in this research.

Table 2. SWOT analysis results. List of helpful and harmful factors regarding the implementation of a mini-round box sampling strategy to monitor orthopteran assemblages in alpine and subalpine grasslands. Following a SWOT framework, strengths and weaknesses are considered as mini-round boxes' internal factors (i.e. intrinsic technical features of the sampling method, positive and negative), while opportunities and threats are external factors (i.e. positive or negative environmental, strategic, and ecological features). The asterisk highlights the points requiring further research.

	Helpful factors	Harmful factors
Internal factors	**Strengths**	**Weaknesses**
	– Handy (small and light) sampling device	– Small sampling unit (0.16 m^2)
	– Inexpensive (< 50 €)	– High number of repetitions per site required
	– Easy and quick use	– Complementary monitoring required
	– One operator needed	– Few methodological literature available
	– Capture method	
	– Standardized sampling unit	
	– Species richness and abundance data	
	– Sex ratio data	
	– Life-history data (adult/immature stages)	
External factors	**Opportunities**	**Threats**
	– Suitability for challenging and/or less accessible environments	– Underestimates in species richness
	– Suitability for inexperienced operators	– Underestimates in population density
	– Suitability for high orthopteran densities	– Biased community composition data
	– Suitability for random sampling	– Lack of comparability among grassland sites
	– Suitability for community ecology	– Lack of comparability with standard box-based samplings (≥ 1 m^2)
	– Independence from grass height*	– Uncertain relationship between population density and species' mobility*
	– Independence from species' mobility*	

affecting orthopteran assemblages (Gardiner 2018). Similarly, the general decrease in vegetation height towards high altitudes (Pellissier et al. 2010) does not affect the mini-round box representativeness. However, this research is not based on a specific and well-balanced sampling design to analyse the grass height effect (i.e. standardized comparison of mini-round box accuracy among different grass height classes). Therefore, further specific analyses are required to confirm these outcomes.

According to the results obtained in this study, an additional opportunity given by the mini-round box method is its independence from orthopteran species' mobility, at least at community level and from a qualitative point of view. In particular, although a bias towards less mobile species was expected in mini-round box samples (i.e. high escape capacity by highly mobile species before the box's drop), no significant differences in terms of mean species mobility index have been found in the comparison with the combined survey outcomes. This may be an advantage if different grassland orthopteran communities have to be qualitatively compared, without any confounding detection effect due to species' dispersal (and escape) capacity. Such positive feature of box-based samplings was already described by Gardiner and Hill (2006) in their comparison between box quadrats, open quadrats and transect counts. Indeed, these authors observed limited orthopteran density underestimates in box quadrats compared with the other two methods, ascribing this difference to the reduced number of individuals able to escape from the box. However, the same authors specify that orthopterans were occasionally observed to escape from the sampling unit as the box quadrat was dropped onto the vegetation. This latter observation highlights a possible additional role of the species-specific escape distance behaviour in conditioning boxes' sampling accuracy, rather than a simple effect of the species' dispersal ability.

Anyway, the alpine and subalpine orthopteran assemblages investigated here are predominantly composed by species with a low to moderate mobility (79.3% of the whole community), less likely to successfully escape to mini-round box samplings. Thus, the effect of species' dispersal capacity on mini-round box sampling accuracy might change in communities where highly mobile species are predominant, perhaps increasing the proportion of undetected taxa. For instance, Fartmann et al. (2024) recommend the implementation of separate transect counts targeted on readily-flying species in order to overcome this issue. Accordingly, this topic needs further research, especially concerning possible biases in box-based population density estimates between mobile and sedentary species.

Unfortunately, the mini-round box sampling strategy involves also a number of harmful factors. Despite its manageability, the small sampling unit area (0.16 m^2) certainly represents a major weakness of this method, producing detrimental effects on the qualitative (and likely quantitative) accuracy of samplings. Indeed, in this study an average of only the 53% of the orthopteran species detected with the combined survey were successfully sampled with mini-round boxes in alpine and subalpine grasslands. This is in accordance with Badenhausser et al. (2009), who proved that box quadrats smaller than 1 m^2 cause underestimates in orthopteran density. As a consequence, the qualitative (and quantitative) data resulting from mini-round box samplings have to be always considered with caution by grassland ecologists, since, as observed in this research, inaccurate outcomes in terms of orthopteran community composition are likely produced. Accordingly, the probability of misleading conclusions in ecological studies based on a mini-round box sampling strategy is high.

A further issue when dealing with mini-round box data concerns the lack of comparability among different

grassland sites. According to the Beta-GLMM results, the mini-round box sampling accuracy proved to significantly change between the two sites investigated in this research (i.e. Troncea Valley and Rocca Bianca), thus making unreliable any ecological comparison among them. Indeed, any site-related response of orthopteran assemblages might be hindered by the differential sampling efficiency, likely leading to misleading conclusions from an ecological point of view (Blaustein and Spencer 2005). Besides the several possible reasons that may explain this outcome, this result represents a serious constraint for the suitability of the mini-round box surveys for large-scale (and long-term) studies, at least in the setting experimented here.

Regrettably, the possible solutions to compensate the mini-round box lack of representativeness cannot be considered as advantageous. The most obvious strategy to improve the mini-round box survey accuracy is to increase the sampling unit area, but losing many of the positive logistic features of this method. For instance, a 1 m^2-round box consists of a cylinder of 112 cm in diameter, rather big to be transported and rapidly thrown into the grass in often steep and uneven alpine and subalpine grasslands. Therefore, a smaller trade-off size to accommodate both sampling accuracy and convenience in such challenging environments is needed, requiring further specific tests in the field to be properly identified and evaluated.

To enhance the sampling effort is another possible solution to improve mini-round boxes accuracy, although it implies additional work and time spent for researchers. A first strategy is to increase the number of sample unit repetitions per site, following the species-sampling effort relationship theory (Gotelli and Colwell 2001; Cam et al. 2002; Azovsky 2011). In this context, important data are provided by Ingrisch and Köhler (1998), who identified an area between 9 and 15 m^2 as minimum sample size per plot to ensure orthopteran species richness saturation with box quadrat surveys in central European grasslands. Similarly, Fartmann et al. (2024) suggest 20 m^2 as minimum sampling area per plot. Given that in this study the overall sampling area per transect is 9.6 m^2 (i.e. 0.16 $m^2 \times 60$), to double the number of mini-round box drops per sampling site (i.e. $n = 120$; sampling area 19.2 m^2) might be an optimal solution to maximise the results from a qualitative point of view. At least, 30 drops per plot should be added to reach an area of 14.4 m^2 per site (i.e. $n = 90$), following Ingrisch and Köhler (1998). However, the feasibility of this option depends on the extent of sampling sites, which in turn is related to the research aims, sampling design, extent of grassland patches, accessibility, etc. For instance, in this study the limited transect length (200 m) constrained the performance of a higher number of independent drops (i.e. enough spaced each other), also compromising the performance of further analysis on the effect of an enhanced number of repetitions on sampling efficiency.

A second choice is to increase the sampling effort by performing a complementary monitoring, to be implemented in parallel with mini-round box samplings, but applying a different survey technique. For instance, timed counts have been used by Marini at al. (2008) and Kurtogullari et al. (2020) to complete their box-based samplings, while in this study the considerable proportion of orthopteran species missed by mini-round boxes (47% in average) was successfully detected by means of a visual and acoustic survey. This result is in accordance with Mourguiart et al. (2020), who confirmed that visual counts maximise orthopteran detectability in alpine and subalpine grasslands, especially when paired with stridulations' listening. Therefore, the simultaneous implementation of a standardized visual and acoustic monitoring might represent a suitable solution to overcome the observed lack of accuracy of mini-round box surveys in the alpine context, although an additional trained operator might be required.

Lastly, an important weakness of the mini-round box method is the lack of scientific literature. In particular, specific methodological papers are unavailable, forcing to a general reference to the box quadrats' literature for technical details (e.g. Gardiner et al. 2005; Badenhausser et al. 2009; Fartmann et al. 2024). In addition, since most of the available research is based on 1 m^2-sampling units, the comparison of mini-round box survey results with other box-based studies may be difficult, thus limiting their potential contribution in wider research in grassland ecology.

In conclusion, a thoughtful evaluation of mini-round boxes' suitability as a sampling method to monitor orthopteran assemblages in alpine and subalpine grasslands is not an easy task. Overall, mini-round boxes show a good potential as a handy, easy, cheap, and standardized sampling method, but serious shortcomings in terms of species detection have to be accounted by ecologists when analysing the resulting data. A number of valuable strengths and interesting opportunities are counteracted by serious weaknesses and significant threats, which need to be carefully evaluated when planning a sampling design. Thanks to the SWOT approach applied in this paper, a clear list and categorization of positive and negative factors resulting from the implementation of this sampling method are provided, hopefully helping grassland ecologists in the selection of the best survey strategy to successfully answer their research questions. In addition, although requiring further experimentation, the proposed solutions to improve the mini-round box accuracy may enhance the value of this method for biodiversity monitoring and ecological studies in alpine grassland habitats, perhaps further encouraging the use of orthopterans as environmental indicators in even challenging ecosystems.

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